REMARKS

Status of the Claims

Claims 39-43 and 45-79 are pending in this application. Claims 39-43 were previously withdrawn from consideration. Claims 45-79 stand rejected. Claim 47 has been cancelled. Claim 45 has been amended to include the phrase “if a fluorescence emission from the first semiconductor nanocrystal occurs.” Support for the amendment may be found on, e.g., page 4 of the specification, as filed, and in original claim 23. This amendment was introduced and discussed in response to the previous Office Action. However, Applicants failed to show the amendment to claim 45 in the Listing of Claims. Applicants wish to thank the Examiner for pointing out this inadvertent omission. Claims 68, 69, 71, 76, and 77 have been amended to more clearly describe the claimed subject matter and to correct inadvertent typographical errors. New claim 80 has been added and finds support on, for example, page 7, lines 7-20 and page 22, lines 11-16 of the specification, as filed.

No new matter is added by the amendments. Reconsideration of the claims in view of the following remarks is respectfully requested.

Claim Rejection under 35 U.S.C. § 103(a)

Claims 45-49, 58-59, 61-63, 67-72 and 74-79 were rejected under 35 U.S.C. 103(a) as allegedly unpatentable over Mirkin et al. (WO 98/04740) in view of Weiss et al. (U.S. Patent No. 5,990,479) and further in view of Pinkel et al (U.S. Patent No. 5,690,894).

The Applicants traverse this rejection and offer the following additional discussion in view of the Response to Arguments in the Office Action.

The Office Action characterizes Mirkin et al. as follows:

> In a single exemplary embodiment, Mirkin et al teach providing a substrate attached to a first target; namely, a substrate comprising a plurality of initial type of oligonucleotides attached to the substrate in an array of spots, wherein each spot contains a different type of oligonucleotide (page 40, line 30-page 41, line 11). The plurality of different initial type of oligonucleotides attached in an array of spots is the claimed plurality of different targets attached to a substrate. Because there are different types of targets,

each target can preferentially bind to a corresponding different probe polynucleotide. The substrate is contacted with the first sample, wherein the first sample is suspected of comprising the first probe; namely, analyte DNA is added to the substrate (Figure 13B); the instantly claimed "first probe" is the analyte DNA of Figure 13B, and the instantly claimed 'targets' are the adsorbed thiol modified DNA of Figure 13B. The first probe comprises a first probe polynucleotide comprising a first tag sequence which does not bind to the first target and a first binding sequence which does bind to the first target, and wherein contacting the substrate with the first sample takes place under conditions in which the first binding sequence can bind to the first target; namely, Figure 13B shows part of the first probe hybridizing to the immobilized targets through a first binding sequence, and the remainder of the first probe is a first tag sequence available to bind to DNA modified nanoparticles. The DNA modified nanoparticles of Figure 13B are the tag-binding conjugate, which binds to the first tag sequence. The nanoparticles are a semiconductor nanoparticles (page 19, lines 24-34), and determining if the semiconductor nanocrystal is associated with the substrate occurs because Mirkin et al teach color changes resulting from binding on the substrate where noted (page 83, lines 18-20 and Figure 13B, last step).

The present rejection is based on the amplification scheme described in Figure 13B, which involves aggregation of gold nanoparticles through hybridizable binding sequences. The purpose of this scheme is to use a combination of hybridizations to induce a detectable change in the mixture upon hybridization of nucleotide sequences to an immobilized target. The scheme relies on amplification of the detectable change using a series of hybridizations, such that the change may be readily observed with the naked eye (see, e.g., page 36, lines 3-11). Aggregation of gold nanoparticles according to the scheme of Figure 13B manifests itself as a "darkened area" on the substrate.

<u>Mirkin does not use a semiconductor nanoparticle.</u>

The Examiner asserts that it would have been obvious to a person of ordinary skill at the time the claimed invention was made to modify the method of Figure 13B to use semiconductor nanoparticles, including the semiconductor materials listed on page 19, lines 24-35. The following three reasons were offered in the Office Action to support the Examiner's position.

1) "The description of Figure 13B on page 17 of Mirkin et al. merely states that the method uses 'nanoparticles' and does not further state that type of material used is limited to gold."

2) "The description of Figure 13B on page 36 states that method illustrated is '[a]n example' and uses 'nanoparticles' and does not further state that type of material used is limited to gold."

> 3) "Example 6, which specifically uses gold nanoparticles, states that the DNA modified nanoparticles were absorbed 'as shown in Figure 13B,' but does not state that Figure 13B is strictly limited to the use of gold nanoparticles."

According to the Examiner, the particles depicted in Figure 13B are not limited to gold nanoparticles and can be replaced with semiconductor nanoparticles. Such a replacement is not justified in view of the combination of cited references.

As mentioned above, Figure 13B (and the associated discussion in Example 6) relate to a colorimetric assay based on <u>amplification of a detectable signal</u>. The assay involves adhering gold nanoparticles to a thiol terminated surface. Thiol-modified DNA is adsorbed onto the particles (considered by the Examiner to be equivalent to the "target" of the instant claims). Analyte DNA strands (considered equivalent to the "first probe" of the instant claims) are hybridized to the DNA modified nanoparticles. The unhybridized portion of the analyte DNA strands (considered equivalent to the "first tag sequence" of the instant claims) are hybridized to DNA modified nanoparticles (considered equivalent to the "tag binding conjugate" of the instant claims). Example 6 clearly identifies the DNA-modified nanoparticles of Figure 13B as "gold nanoparticles" (see, page 73, lines 32-33 and page 74, lines 7-9). Figure 13B further shows that the hybridization sequence can be repeated to further amplify the detectable signal. The combination of hybridizations produces a "darkened purple color" where nanoparticle aggregates are linked to the substrate that can be readily observed with the naked eye (see, page 36, lines 3-11 and Figure 13B caption). Thus, contrary to the Examiner's assertion, the description of Figure 13B clearly limits the depicted nanoparticles to *gold nanoparticles*.

The Examiner indicates that semiconductor nanoparticles have been disclosed in Mirkin et al. as an alternative to gold nanoparticles. Therefore, Figure 13B is to be broadly interpreted to include semiconductor materials. Further, semiconductor nanoparticles may be spectroscopically detected. The Examiner also contends that no motivation is necessary to make the suggested substitution. According to the Office Action,

> "Mirkin et al. <u>**by itself**</u> actually contemplates the use of semiconductor nanoparticles and spectroscopic detection of oligonucleotide nanoparticle complexes. Because the prior art of Mirkin et al. <u>**by itself**</u> contemplates the use of semiconductor nanoparticles and spectroscopic detection of oligonucleotide nanoparticle complexes, no motivation is necessary."

The Applicants disagree that Mirkin et al. contemplated the use of semiconductor nanocrystals in the assay depicted in Figure 13B. No concrete explanation has been provided as to *why* a person of skill in the art would have been motivated to replace the preferred gold nanoparticle of Example 6 with a semiconductor nanoparticle. Also no reasons have been provided as to why spectroscopic detection should be used in the Example when the Mirkin assay is clearly directed to visualization with the naked eye. As pointed out previously, Figure 13B is directed exclusively to gold nanoparticles. Mirkin et al. emphasizes throughout the specification that gold nanoparticles are preferred and allow for visualization with the naked eye. Although Mirkin et al. disclose semiconductor nanoparticles generally, no basis for substituting gold nanoparticles with semiconductor nanoparticles in the assay depicted in Figure 13B has been provided either by the reference itself or the Examiner. Mirkin et al. does not equate these two types of materials or suggest that they can be interchangeably used in the assay of Figure 13B. The Weiss et al. and Pinkel et al. references are silent on this matter, as well. The ordinary artisan would also know from common general knowledge that gold is not a semiconductive material and, therefore, would not be considered equivalent or interchangeable with a semiconductive nanoparticle. In the absence of any teaching to suggest that gold and semiconductor nanoparticles could be used interchangeably to effect signal amplification, a person of ordinary skill in the art would not consider substituting gold nanoparticles with semiconductor nanoparticles in the assay of Figure 13B. Furthermore, just because such a substitution conceivably *could* be made based on the fact that Mirkin et al. discloses semiconductor nanoparticles generally, does not mean that the ordinary artisan *would* have made that substitution in light of the obvious preference for gold particles emphasized repeatedly in Mirkin et al. The substitution proposed by the Examiner would have been inconsistent with the reference's stated preference for gold nanoparticles. Further, such a replacement would be unwarranted and would unnecessarily complicate the assay.

<u>No reason to modify Mirkin et al. in view of Weiss et al.</u>

Weiss et al. has been cited to show that biological molecules can be attached to semiconductor nanocrystals and that such constructs can be *simultaneously detected* due to the emission properties of the nanocrystals. The Examiner contends that a person of ordinary skill would have had motivation to modify Mirkin's examples in view of the teachings in Weiss et al. According to the Office Action, the "modification would have resulted in allowing simultaneous

detection of a plurality of detectable substances without overlap as explicitly taught by Weiss et al. (column 6, lines 35-47)' (page 6 of the Office Action). The Examiner concludes that it would have been obvious to modify the method of Mirkin et al. in view of the disclosure of Weiss et al. to arrive at the instantly claimed method with a reasonable expectation of success. The Applicants respectfully disagree with this conclusion.

Mirkin's failure to describe separate detection of multiple binding events represents a fundamental difference between Mirkin's disclosure and the present claims. The tag-binding conjugates in the present claims are designed for selective binding of distinct nanocrystals to different probe polynucleotides, thereby permitting simultaneous interrogation of large numbers of different probe polynucleotides and targets. In particular, the instant claims require the use of a different tag-binding conjugate that binds to each different probe polynucleotide, wherein each different tag-binding conjugate comprises a different semiconductor nanocrystal with different fluorescence characteristics. The assay of Figure 13B, in contrast, uses only one type of nanoparticle (i.e., gold). Use of a single type of nanoparticle is consistent with the objective of the assay. The assay's objective is to use the *same* type of nanoparticle to *amplify* signal resulting from a hybridization event. The signal to be detected in the assay results from the cumulative effect of having many, identical particles (with the same optical properties) agglomerate in a localized area due to multiple hybridization events. The Examiner proposes that it would have been obvious to use different types of semiconductor nanocrystals (as disclosed in Weiss et al.) in the assay of Figure 13B. Different types of nanocrystals have different fluorescence characteristics. For example, nanocrystals of different sizes are known to emit at different wavelengths. The use of different types of nanocrystals (where each type possesses different fluorescence characteristics) in the Figure 13B assay would not achieve the cumulative effect needed to generate an amplified detectable signal. Consequently, the use of different types of nanocrystals in the Mirkin method would compromise the outcome of the assay of Figure 13B. Accordingly, the modification suggested by the Examiner changes Mirkin's mode of operation, which is impermissible in an obviousness rejection (see, M.P.E.P. § 2143.01(VI)).

<u>No reason to modify Mirkin et al. and Weiss et al. in view of Pinkel et al.</u>

Pinkel et al. is relied upon for teaching *separately determining* each binding event on an array. According to the Office Action, Pinkel et al. teaches "a method of assaying samples for probes by using a biosensor array to detect nucleic acid binding complexes (Abstract), wherein each binding event is separately determined." Further, the Office Action claims that an ordinary artisan would have been motivated to apply the method of separate determination of each binding event on an array of Pinkel et al. to the method of Mirkin et al. in view of Weiss et al. with predictable results.

The abstract of Pinkel is a generalized statement and does not mention "using a biosensor array to detect nucleic acid binding complexes." In general, the abstract discloses several examples of various types of binding complexes that can be used with the optical fiber biosensors disclosed in Pinkel et al. Despite the Examiner's assertion to the contrary, the optical fiber array of Pinkel et al. does not relate to the field of nanocrystals and cannot, therefore, be considered analogous art. Furthermore, whether or not Pinkel et al. discloses separate determination of binding events is irrelevant, since the Pinkel et al. disclosure fails to remedy the deficiencies of the Mirkin and Weiss references.

Accordingly, it would not have been obvious to combine the methods described in Weiss and Mirkin with the method described in Pinkel. Withdrawal of the rejection is respectfully requested.

<u>Rejections of dependent claims</u>

The rejection of claim 45 is overcome for the reasons presented above. All of the other claims under examination depend from and thus include all limitations of claim 45. All of the rejections of these dependent claims rely upon the above-discussed rejection of claim 45 based on the combination of Mirkin et al., Weiss et al., and Pinkel et al. Therefore, all of the rejections of claims depending from claim 45 are also overcome. These claims are allowable over the cited art because they include all limitations of a claim that is allowable over the cited art, and the rejection presented no basis to show that the additional references cited overcome the deficiencies noted above with regard to the characterization and application of Mirkin et al., Weiss et al., and Pinkel et al to claim 45.

For the reasons set forth above, all claims under examination are believed to be allowable. Reconsideration and withdrawal of those rejections are respectfully requested.

CONCLUSION

In view of the above remarks, it is submitted that this application is now ready for allowance. Early notice to this effect is solicited. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (541)-335-0070.

Respectfully submitted,

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/Karen B. Geahigan/
Karen B. Geahigan, Reg. No. 52,936

Life Technologies Corporation
Customer No. 52059
Phone: (541) 335-0070
Facsimile: (541) 335-0234